Species	Number	Counted by	Collection	Locality
frutescens (L.) DC.	2n = 28	R. T. Neher IND	Heiser *3205* IND	Manatee County, Florida
Simsia				
grandiflora Benth.	n = 17	C. B. Heiser IND	Heiser *R7* IND	San Salvador, Salvador
polycephala Benth.	n = 17	C. B. Heiser IND	Heiser *R5* IND	Antigua, Guatemala
Thelesperma				
intermedium Rydb.	n = 8	A. M. Torres UNM	Torres *12* UNM	Bernalillo County, New Mexico
longipes Gray	n = 10	A. M. Torres UNM	Torres *18* UNM	Otero County, New Mexico
megapotamicum (Spreng.) Kuntze	n = 11	A. M. Torres UNM	Torres *10* UNM	Socorro County, New Mexico
subnudum Gray	n = 12	A. M. Torres UNM	Torres *23* UNM	San Juan County, New Mexico

APOMIXIS IN THE GRAMINEAE. TRIBE ANDROPOGONEAE: HETEROPOGON CONTORTUS

W. H. P. Emery and W. V. Brown

Heteropogon contortus (L.) Beauv. ex Roem. & Schult. consists of a relatively uniform series of populations with an extensive native range throughout most of the tropical and sub-tropical grassland regions of the world. In parts of the Hawaiian Islands, Australia, Indo-Malaya, India, Asia Minor, Africa, Europe, and the Americas it forms an important part of the range forage. The species is both palatable and nutritious, but when mature the plants produce fertile spikelets which have a sharply pointed callus and a stout hairy awn. These spikelets may penetrate the skin or lining of the digestive tract (Pammel, 1911), causing severe irritation and infection. They may even affect the general health of grazing animals (Chippindall, 1954).

Previous cytological studies of *H. contortus* from various parts of the world have shown that many of the populations which comprise this species are characterized by highly irregular meiotic divisions. Gould (1956) reported that some irregularities were observed in meiotic divisions of the pollen mother cells (PMC's) in six collections from Texas and northern Mexico. Mehra (1954) examined six collections from India and reported varying numbers of univalent, bivalent, trivalent, and quadrivalent configurations in the microsporocytes of each collection. On the other hand, Celarier and Harlan (1953) examined collections from Tanganyika, India, Australia, and Madagascar and noted a high degree of irregularity in the

PMC's of the first three, but an almost regular division in the PMC's of the collection from Madagascar.

Evidence for the existence within the species of a polyploid series, with occasional aneuploid forms, appears well-substantiated by a number of workers (Brown, 1951; Celarier and Harlan, 1954; Darlington and Janaki-Ammal, 1946; de Wet, 1956; Gould, 1956; and Mehra, 1954). The chromosome counts reported in 22 different collections of *H. contortus* range from 2n=20 to 2n=80, with modes at 2n=40 and 2n=60. The irregularity of meiotic divisions, and the existence of an extensive polyploid series of chromosome forms, suggested the possibility of apomixis in this species.

Materials and Methods

Seed from thirty collections were obtained through the generous cooperation of Dr. R. P. Celarier of the Oklahoma Agricultural Experiment Station and Dr. E. J. Britten of the Hawaii Agricultural Experiment Station. Flowering material of twelve American collections was provided through the cooperation of Dr. F. W. Gould of Texas Agricultural and

Table 1. Cultures of Heteropogon Contortus Examined[1]

Culture No.	Place of Origin	2n	Culture No.	Place of Origin	2n
A-4595-1	Galton, Australia	....	A-3729-4	Southern Rhodesia	....
A-4595-3	Galton, Australia	....	A-3050-2	Madagascar	40
A-3962	Borgar, Java	40	A-3050-3	Madagascar	40
A-2667	New Delhi, India	40	G-683	Monterrey, Mexico	60
A-5293	Delhi, India	....	G-730	Durango, Mexico	...
A-5298	India	....	G-788	Parral, Mexico	....
A-2668	Coimbatore, India	40	G-847	Chihuahua, Mexico	60
A-3230-3	Allahabad, India	40	G-420	Cuauhtemoc, Mexico	....
A-4829	Dehra Dun, India	....	G-384	Chihuahua, Mexico	60
A-3703-1	Belgian Congo	40	G-500	Durango, Mexico	....
A-3703-2	Belgian Congo	40	G-1485	Durango, Mexico	....
A-3703-3	Belgian Congo	40	G-1556	Coahuila, Mexico	....
A-3234	Kenya	40	G-874	Encino, Texas	....
A-3729-1	Southern Rhodesia	....	G-873	Encino, Texas	....
A-3729-3	Southern Rhodesia	....	....	Artesia Wells, Texas	....
			....	Marathon, Texas	....

[1] Voucher specimens for all collections utilized in this study will be deposited in the University of Texas Herbarium. Collections bearing the prefix "A" are also deposited in the Oklahoma State University Herbarium, Stillwater, Oklahoma, and were obtained through the cooperation of that institution. Those with the prefix "G" were provided by the Texas Agricultural and Mechanical College. The plants from Artesia Wells and Marathon, Texas, are collections of the authors.

Mechanical College. The authors wish to express their thanks to these men, as well as to all others who were instrumental in assembling seed from the foreign collections of this species.

Plants of fifteen non-American cultures (7 Indian, 4 African, 1 from Madagascar, 2 Australian, and 1 from Java) were established in our grass nursery together with two collections from Texas.

Collections utilized in the embryological examination of *H. contortus,* together with their place of origin, are given in Table 1. Flowering material at various stages of development was killed and fixed in Navashin's fluid. Pistils were dissected from the florets, dehydrated in butyl-ethyl alcohol, embedded in Tissuemat, sectioned at 8 to 12 microns, and stained in Delafield's hematoxylin.

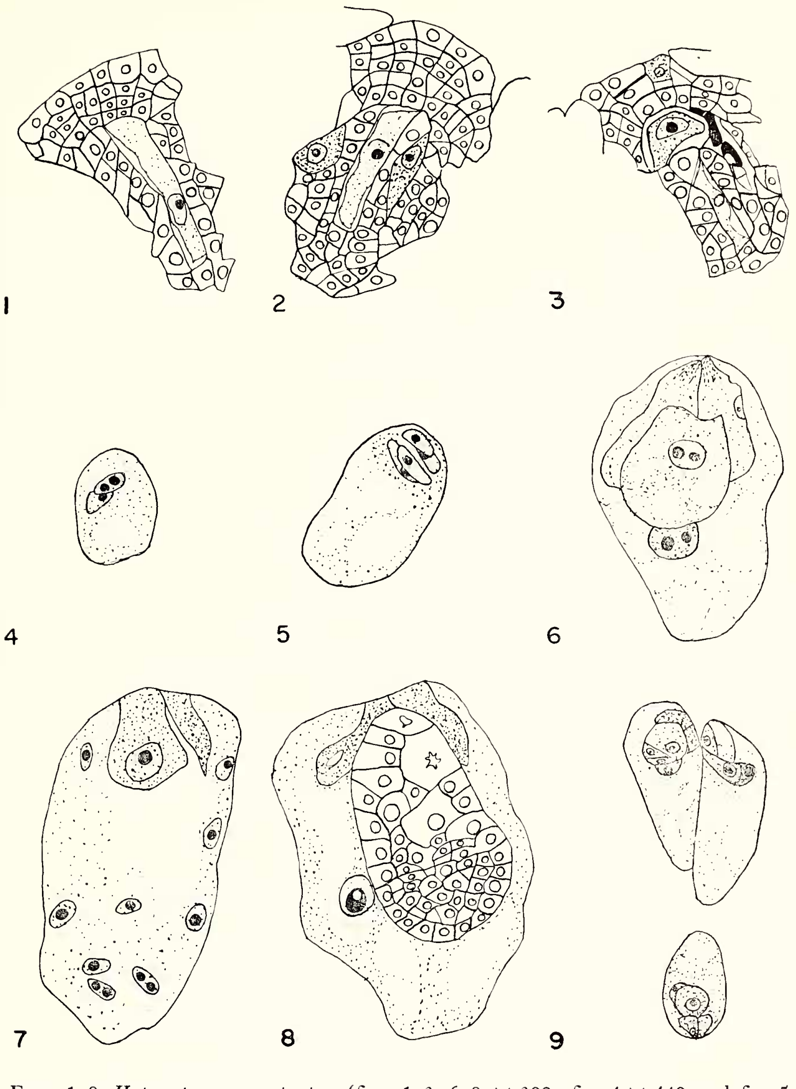
Observations

All thirty-one plants from twenty-five different collections of *H. contortus* examined in this study were found to be aposporous apomicts. The apomictic mechanism involves the regular degeneration of the egg mother cell (EMC) (figs. 1, 2) not later than early prophase of meiosis, and the simultaneous or subsequent development of aposporous embryo sac initials (figs. 2, 3). No EMC division nor derivatives from such a division were observed in this study. The degeneration of the EMC appears to be dependent on factors inherent within the cell, since in many ovules degeneration occurs even in the absence of aposporous embryo sac initials (fig. 1). The EMC becomes less turgid, the cytoplasm becomes more granular and lighter staining. Finally the cell degenerates completely.

Following a period of enlargement and vacuolization, the nucleus of the aposporous embryo sac initial divides twice in rapid succession to produce four nuclei. These nuclei commonly remain in the micropylar end of the embryo sac (figs. 4, 5). As development of an embryo sac proceeds, the four nuclei usually organize into 4-nucleate, 4-celled embryo sacs, with 1 egg, 2 synergids, and 1 polar nucleus (fig. 6). Occasionally, however, 4-nucleate, 3-celled embryo sacs were observed containing 1 egg, 1 synergid, and 2 polar nuclei. No 8-nucleate embryo sacs were observed in the present material.

Many ovules of *H. contortus* produce only one aposporous embryo sac. In a majority of the ovules, however, two or more aposporous embryo sacs commonly develop. These embryo sacs appear to compete for the available space or nutritive supply, enlarging and encroaching upon each other as they develop.

The development of the embryo and endosperm begins shortly after, but apparently never prior to, anthesis. It was not determined if pollination is a prerequisite to development, but the appearance of pollen tube remnants in embryo sacs shortly after anthesis together with the subsequent development of embryos and endosperm strongly suggests that *H. contortus* is pseudogamous. Commonly the endosperm undergoes a num-



FIGS. 1–9. *Heteropogon contortus* (figs. 1–3, 6–9, × 300; fig. 4 × 440 and fig. 5, × 100): 1, egg mother cell (EMC) starting to degenerate; 2, partially degenerate EMC and two developing aposporous embryo sac initials; 3, developing aposporous embryo sac initial at the apex of the nucellus and a portion of the degenerating EMC; 4, two-nucleate aposporous embryo sac with both nuclei in one end of the embryo sac; 5, the four-nucleate aposporous embryo sac; 6, a four-nucleate aposporous emrbyo sac organized with one egg, two synergids, and a single polar nucleus; 7, embryo sac showing the early development of endosperm prior to the initial division of the unreduced egg cell; 8, several-celled embryo and an undivided polar nucleus; 9, three aposporous embryo sacs of the same ovule showing development of a small mature four-nucleate sac in the chalazal region separated from the other two by several layers of nucellar tissue.

ber of divisions before the initial transverse division of the unreduced egg cell (fig. 7), but this pattern of development varies even within different ovules of the same inflorescence. Not infrequently embryos of 32–64 cells were observed "capped" at their apex by an undivided crescent-shaped, polar nucleus (fig. 8).

Although a majority of the aposporous embryo sacs develop in contact and later encroach upon one another, occasional ovules with multiple embryo sacs were observed in which the developing aposporous embryo sacs are relatively small, are separated by nucellar tissue, and develop to maturity without being encroached upon by other embryo sacs of the ovule (fig. 9).

The apomictic mechanism in *H. contortus* is one involving the regular production of 4-nucleate, aposporous embryo sacs followed by parthenogenesis. The complete failure of meiosis in megasporocytes of this species indicates that *H. contortus* is, or closely approaches, the condition of an obligate apomict.

Discussion

Heteropogon contortus is another species in the rapidly increasing list of grasses known to reproduce in whole or in part by agamospermy. *Heteropogon contortus* seems to be essentially an obligate apomict throughout its range, forming 4-nucleate, aposporous embryo sacs followed by parthenogenesis. It is obvious that, although *H. contortus* seems to be essentially an obligate apomict, variation within the species does arise, possibly through occasional hybridization, polyploidy, or chromosome loss. The occurrence of various chromosome forms, together with univalent and multivalent chromosome configurations in the microsporocytes, tends to indicate the probability of some intra- or inter-specific hybridization at one or more periods during the existence of the taxon. The possibility of sexual recombination during recent times is further suggested by the existence of diploid plants of *H. contortus* in India (Janaki-Ammal, 1946). Diploid relatives of aposporous apomicts are almost without exception sexual (Gustafsson, 1947).

The presence of diploid *H. contortus* in India, the fact that most Old World plants of the species have $2n = 40$ and $2n = 60$, and that all New World plants have $2n = 60$ or above would indicate that southern Asia might be the center of origin of the species. Southern Asia is considered to be the center of origin of the tribe Andropogoneae (Hartley, 1950). An Old World origin for the tribe is further indicated by the existence of numerous genera in the Old World that do not occur in the New, whereas there are no genera of New World Andropogoneae not present also in the Old World.

In most details the apomictic process in *H. contortus* is very similar to that reported by the authors (Brown and Emery, 1957) in *Themeda triandra* and *Bothriochloa ischaemum* of the Andropogoneae. The process is also similar to that reported in species from various genera of the Paniceae, i.e., *Cenchrus* (Snyder, 1955), *Panicum* (Warmke, 1954), *Paspalum* (Smith, 1948), *Pennisetum* (Narayan, 1951), *Setaria* and *Urochloa* (Emery, 1957).

In both tribes (except, perhaps, for *Tripsacum* and *Saccharum*) 4-nucleate aposporous embryo sacs are produced following the degeneration of the sporogenous tissue. Pseudogamy appears to be characteristic of all apomicts in these two tribes. A minor difference in the process as it occurs in the two tribes seems to be the time of degeneration of the sporogenous tissues. In the Paniceae degeneration occurs during or just following the formation of megaspores. In the Andropogoneae the megasporocyte degenerates not later than early prophase of meiosis.

There are a number of aposporous grasses in other tribes of the family, but the process in them differs significantly from that in species of the Andropogoneae and Paniceae. In *Bouteloua curtipendula* (Chlorideae) four megaspores are produced by a very irregular apomeiotic division. The chalazal spore enlarges somewhat before it, like the other three spores, degenerates. The aposporous embryo sac is 8-nucleate (Brayant, 1952). In aposporous *Poa* species (Festuceae) the magasporocyte may complete meiosis and may form a functional reduced embryo sac. Whenever aposporous embryo sac initials are formed, however, they inhibit to some extent further development of the megasporocyte or spores. The aposporous embryo sacs produced are usually 8-nucleate when, and if, they reach maturity (Nygren, 1950). As far as known, therefore, the regular formation of 4-nucleate aposporous embryo sacs is restricted to the apomictic species of the Paniceae and Andropogoneae.

Heteropogon contortus is one of the very few grasses supposedly native to both the Old and New World tropics. Although varying greatly in the frequency of occurrence, the distribution of *H. contortus* is reported from every major land mass between 35°N. latitude and 35°S. latitude, and so far as can be determined from existing botanical literature, it is endemic in the New World, and in the Old World from South Africa to Australia, and numerous islands of the Pacific Ocean. The similarity of the apomictic process in clones from different parts of the species range suggests a common origin of the apomictic mechanism previous to the spread of the species throughout its present pan-tropical distribution. The alternative possibility is that apospory has arisen two or more times in isolated populations of the species and subsequently has developed to the same stage in each.

It is interesting to speculate on the possible age of apomixis in *H. contortus* since here is a highly successful, essentially obligate apomictic species, capable of establishing itself in climax grasslands of many regions. Parallel evolution of identical mechanisms for reproduction in various discontinuous and completely isolated populations seems unlikely in such a morphologically uniform species, and thus it may be concluded that the development of apomixis in this species predates its transoceanic distribution.

If the pantropical distribution of *H. contortus* occurred in recent times, then the development of the apomictic mechanism in this species may also be a recent occurrence. If, on the other hand, its distribution occurred together with other tropical and sub-tropical floras, then the development of the apomictic mechanism may date back to the end of the Eocene epoch or earlier. The latter possibility would imply a very old species that, in spite of essentially obligate asexual reproduction, has spread widely and survived despite competition with sexually reproducing species in a variety of environments. There is no documentary evidence known to the authors indicating post-Columbian transfer from the Old World to the New or vice versa. Nevertheless, *Heteropogon* is one of the most highly specialized genera of the Andropogoneae and has two pan-tropical species. It is possible that its highly specialized spikelets, with their sharply barbed callus, may have permitted a recent pre-Columbian intercontinental migration.

In his extensive treatise on apomixis, Gustafsson (1947, p. 303) points out that the phytogeographical data on a number of distinct apomictic micro-species of glaciated regions seems definitely to prove the Old-Quaternary age of such taxa, and therefore, that the condition of agamospermy itself must have arisen in even more remote times. Apomixis in these species has existed, therefore, for somewhat more than one million years. This is the oldest estimate on the age of apomixis in extant species known to the authors, although Clausen (1954) states, "The facultative apomicts have exploited an extremely effective solution to these contrasting demands (referring to environment and species variability), a solution that enables them to store interspecific variability and to nevertheless remain constant for ages."

It appears reasonable that if apomictic microspecies of *Taraxacum*, *Hieracium*, and *Alchemilla* (cited by Gustafsson, 1947) have existed since the late Tertiary in a region of considerable environmental instability, then a definite possibility exists that an essentially obligate apomictic species might have endured under the more stable tropical and sub-tropical environments for a much greater period of time. The duration of apomixis in *Heteropogon contortus* may be short or very long; for the present it remains in the realm of speculation.

Summary

1. Cytological studies of *Heteropogon contortus* (L.) Beauv. ex Roem. and Schult., a perennial forage range grass with an extensive range throughout most of the tropical and sub-tropical grassland regions of the world, revealed this species to be an essentially obligate, aposporous apomict with probable pseudogamous development of embryos.

2. The apomictic mechanism, as studied in twenty-five collections from various parts of the Old and New worlds, involves the regular degenera-

tion of the egg mother cell, initiation of one to several aposporous embryo sac initials, and, following two nuclear divisions in each sac, the organization of 4-nucleate aposporous type embryo sacs, these commonly with one egg, two synergids, and a single polar nucleus. Development of the embryo is probably pseudogamous, but is not dependent on the prior development of endosperm.

3· The close parallel between the apomictic mechanisms of *H. contortus, Bothriochloa ischaemum,* and *Themeda triandra* of the Andropogoneae and the reproductive mechanisms of previously reported aposporous apomicts of the Paniceae is pointed out.

4. Parallel evolution of identical apomictic mechanisms for reproduction in various discontinuous populations seems unlikely, and the authors conclude that the age of apomixis in *H. contortus* predates its trans-oceanic distribution. If this distribution were recent, then the age of apomixis in the species may be recent; but if the species were distributed as part of a tropical or a sub-tropical flora, then the age of apomixis may date back to the end of the Eocene epoch or earlier.

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IRIS, SECTION APOGON, SUBSECTION OREGONAE SUBSECT. NOV.[1]

Quentin D. Clarkson

Iris tenuis Wats. is endemic to the upper Clackamas River and its tributary, Eagle Creek, in Clackamas County, northwest Oregon. A single specimen collected in 1884 from Washington County, Oregon, is apparently mislabeled, as the species has not been collected since from that area.

While the specific validity of *I. tenuis* has not been questioned, it has been included by authors in subsection *Californicae* of section *Apogon* only with some reluctance. Foster (1937) called attention to its morphological and cytological distinctions, but left it in the *Californicae*. Simonet (1934) placed the species in the *Sibiricae* mainly on cytological grounds, but Foster rejected this treatment for morphological reasons. Smith and Clarkson (1956) on the basis of cytological data proposed its removal from the *Californicae*.

Simonet reported a chromosome number of n=14 for *I. tenuis*. Smith and Clarkson confirmed this number and also reported that fertile hybrids between other members of the *Californicae* are easily produced, but that hybrids could not be produced between *I. tenuis* and *I. tenax* Dougl. Presumably this barrier extends to other members of the subsection. Morphologically *I. tenuis* differs distinctively from other *Californicae* in having ensiform leaves and scarious instead of green bracts. Superficially, as Foster pointed out, it resembles *I. cristata* Ait., and the general appearance of the species is unlike other *Californicae*.

Inclusion with the *Sibiricae*, as proposed by Simonet, seems unwise not only because of morphological differences but also because of geographical considerations. If *I. tenuis* is placed with the *Sibiricae* it becomes an isolated member of the subsection with little in common except a possible base chromosome number of n=7.

[1] Grateful acknowledgment is made to Sister John Mary of Marylhurst College for her assistance with the Latin diagnosis and to Dr. Helen Gilkey of Oregon State College for her helpful comments.